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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Mountz, *et al.*	§	ART UNIT: 1647
	§	
FILED: May 15, 1998	§	
	§	
SERIAL NO.: 09/079,834	§	EXAMINER:
	§	Spector, Lorraine
FOR: Fas Ligand Expressing Antigen Presenting Cells for Tolerance Induction	§	
	§	
	§	DOCKET: D6005

#25
D.Q.
7/3/02

The Assistant Commissioner of Patents
BOX RCE
Washington, DC 20231

DECLARATION UNDER 37 C.F.R. § 1.132

Dear Sir:

I, John D. Mountz, does hereby state as follows:

I am a co-inventor of the above-referenced patent application. I have read U.S. patent application serial no. 09/079,834 and I am aware of the content of the Office Action, including all prior art cited against the '834 application.

An issue relating to the patentability of methods of using Fas ligand-expressing antigen presenting cells to induce systemic tolerance to an antigen is the degree of enablement provided by Applicants' specification. The following data are presented as evidence of enablement commensurate with the scope of the claims. The experiments described below tested the therapeutic efficacy of antigen presenting cells that have been modified to express high levels of Fas ligand (APC-FasL) in murine models of Sjögren syndrome–like disease and arthritis in FasL-deficient *gld* mice after infection with murine cytomegalovirus (MCMV) and *M. pulmonis*, respectively. The results showed dramatic amelioration of these diseases in infected *gld* mice.

FasL-deficient B6-*gld/gld* mice infected with murine cytomegalovirus exhibited normal clearance of murine cytomegalovirus from the lung, kidney and liver within 2 weeks of infection. However, an inflammatory response persisted in these organs for over 8 weeks, with a chronically increased T cell response to murine cytomegalovirus-infected APCs and production of autoantibodies. Administration of APC-AdFasL at 4 weeks resulted in down modulation of the inflammatory response in the lungs, liver, and kidney (Figure 1), together with a reduction in the T cell response (Figure 2) and in autoantibody production (Figure 3). APC-AdFasL that had been transfected with UV-irradiated murine cytomegalovirus were more effective than uninfected APC-AdFasL in ameliorating the chronic inflammation. The APC-AdFasL migrated preferentially to the spleen where they triggered apoptosis of lymphocytes in the marginal zone of the spleen. These results confirm that Fas-mediated apoptosis is required for down modulation of the virally induced chronic inflammatory response. This organ-wide effect is mediated by APC-AdFasL-mediated elimination of the activated T lymphocytes in the spleen prior to their emigration to the target organs.

Sjögren syndrome is a chronic inflammatory disease of unknown etiology characterized by infiltration of the exocrine glands with mononuclear cells, predominantly T cells, leading to a sicca syndrome owing to acinar and ductal gland destruction. Infection of B6-*gld/gld* mice with murine cytomegalovirus resulted in development of chronic sialadenitis after clearance of murine cytomegalovirus similar to that observed in human Sjögren syndrome. Using this model for Sjögren syndrome, it was determined whether local FasL expression using recombinant adenoviral vectors might affect the severity of sialadenitis. High levels of FasL expression were achieved by

direct injection of *lpr*-APC-AdFasL into salivary glands to avoid the risk of systemic toxicity. Treatment of acute and chronic sialadenitis in B6-*gld/gld* mice 28 and 75 d following murine cytomegalovirus infection with *lpr*-APC-AdFasL resulted in a significant reduction in size and number of inflammatory foci in salivary gland tissue compared with *lpr*-APC-AdLacZ-treated mice. Hence, using the new model of Sjögren syndrome and local FasL gene transfer, it is demonstrated that infiltrating mononuclear cells were highly susceptible to Fas/FasL-induced apoptosis whereas the ductal and acinar cells were not sensitive to FasL-mediated cell death. These data support a pathogenic model of Sjögren syndrome in which decreased apoptosis of activated T cells leads to increased accumulation of these cells to induce chronic sialadenitis and the subsequent destruction of salivary glands. Induction of FasL expression by the salivary gland parenchymal cells eliminates the activated T cells without causing cytotoxicity to the salivary gland parenchymal cells.

These results indicate that chronic inflammation in specific sites may result from the combined effect of initial viral tropism and inflammation at these sites and failure to eliminate T cells at these sites because of a defect in Fas-FasL interaction. This was further tested in *lpr* and *gld* mice after infection with *M. pulmonis*. *M. pulmonis* infection targets the lung, spleen, and joints of these mice. Similar to infection with murine cytomegalovirus, there was normal clearance of *M. pulmonis* from the lung, spleen, and joints of *lpr* and *gld* mice. However, there was a chronic inflammation that was most pronounced in the joints of these mice, but not in wild-type mice. This chronic inflammation led to a destructive arthritis and development of aggressive synovial fibroblast that eroded and invaded joint cartilage and bone.

M. pulmonis–infected *gld* mice were treated with five doses of *lpr*-APC-AdFasL injected i.p. to determine its systemic effect for preventing the development of post-mycoplasma-induced arthritis. The results showed that there was no significant increase in the number of apoptosis or synovial cells or infiltrating cells in the joint; however, treatment with *lpr*-APC-AdFasL eliminated lymph node T cells and ameliorated the development of postmycoplasma infection–triggered arthritis (Figure 4). These results indicate that defective deletion of activated T cells triggered by *M. pulmonis* infection is an important pathogenic mechanism for the induction of *M. pulmonis*–induced murine arthritis. Furthermore, unlike local treatment strategy described in the murine cytomegalovirus murine Sjögren syndrome model, the present results suggest that elimination of T cells in the lymphoid organ before their emigration to the target organs can also be effective in preventing acute and chronic arthritis triggered by infectious pathogens.

Based on the data contained herein, I respectfully submit that the scope of methods of using Fas ligand-expressing antigen presenting cells to induce systemic tolerance in the '834 application has a reasonable correlation to the scope of the enablement provided.

Figure Legends

Figure 1 shows that there was less severe inflammation in the lung, kidney and liver of APC-AdFasL treated MCMV-infected B6-gld/gld mice. B6-gld/gld mice were treated with either APC-AdCMVLacZ (control), APC-AdFasL or MCMV-APC-AdFasL beginning 28 d after MCMV infection. The mice were treated every 3 d with 4

doses and analyzed 4 wk after initiation of APC therapy. There were at least five mice in each treatment group. The lung, kidney and liver were stained for H & E and the severity of inflammation and tissue damage were scored by three blinded investigators on a relative scale ranging from 0 (not present) to 4+ (most severe). At least 10 fields of view were analyzed for each organ of each mice. The bar graph represents the mean +/- SEM of the inflammatory scores from each organ for mice undergoing the indicated treatment. * indicates values that were significantly different compared to the control treatment group ($p<0.05$).

Figure 2 shows the percentage of murine cytomegalovirus -reactive T cells in vivo. Spleen cells were isolated from murine cytomegalovirus infected mice 4 weeks after treatment with APC-AdLacZ control, APC-AdFasL, or murine cytomegalovirus-infected APC-AdFasL. T cells were stimulated in vitro with murine cytomegalovirus pulse APCs from B6-lpr/lpr mice, and the supernate was harvested 48 h later. * indicates values that were significantly different compared to the control treatment group ($p<0.05$).

Figure 3 shows decreased autoantibody production after treatment with APC-AdFasL. Four weeks after infection with murine cytomegalovirus, mice were treated with APC-AdLacZ control, APC-AdFasL or murine cytomegalovirus-infected APC-AdFasL and sera was collected 4 wk later. Production of anti-dsDNA and RF was determined on d28 post-murine cytomegalovirus infection of B6-gld/gld mice. * indicates values that were significantly different compared to the control treatment group ($p<0.05$).

Figure 4 shows the development of post *M. pulmonis* infection–induced acute lymphocytic infiltration and chronic arthritis in *gld* mice was markedly ameliorated

by *lpr*-APC-AdFasL treatment. Top panel shows knee joints of *gld* mice treated with *lpr*-APC-AdLacZ. Infection of FasL-defective *gld* mice with *M. pulmonis* resulted in severe, acute (1 week postinfection) lymphocytic infiltration and chronic (8 week postinfection) destructive arthritis and development of aggressive synovial fibroblast that eroded and invaded joint cartilage and bone. Bottom panel show knee joints of *gld* mice treated with *lpr*-APC-AdFasL (magnification = X80). Administration of *lpr*-APC-AdFasL to *M. pulmonis*-infected *gld* mice effectively reduced acute lymphocytic infiltration and chronic destructive arthritis.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or patent issued thereon.

Date: 6-13-02

Dr. John D. Mountz

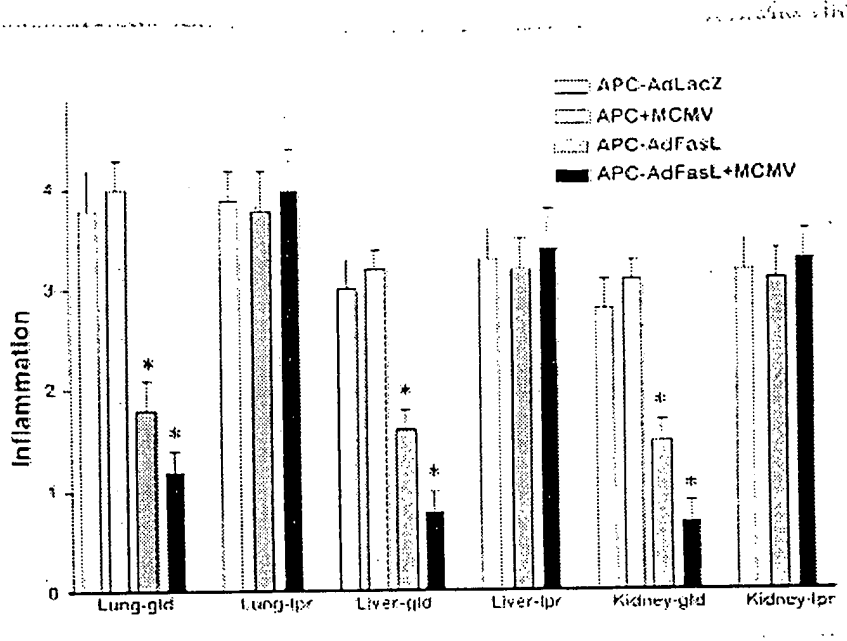

Figure 1

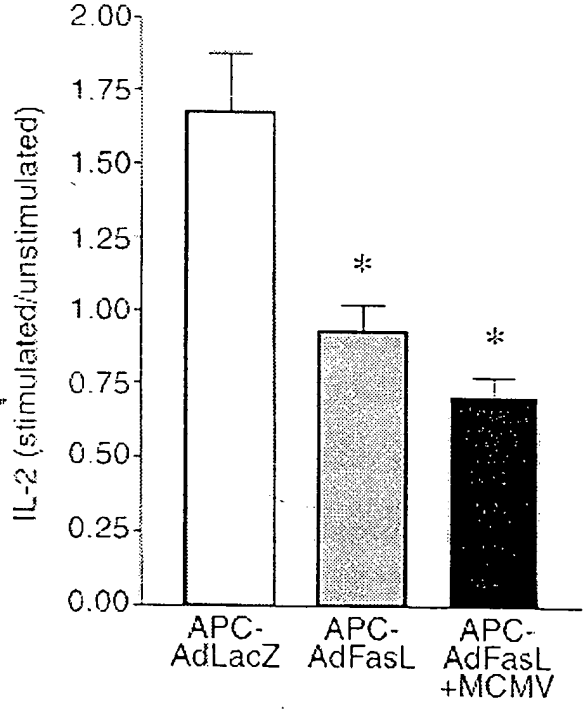

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Figure 2

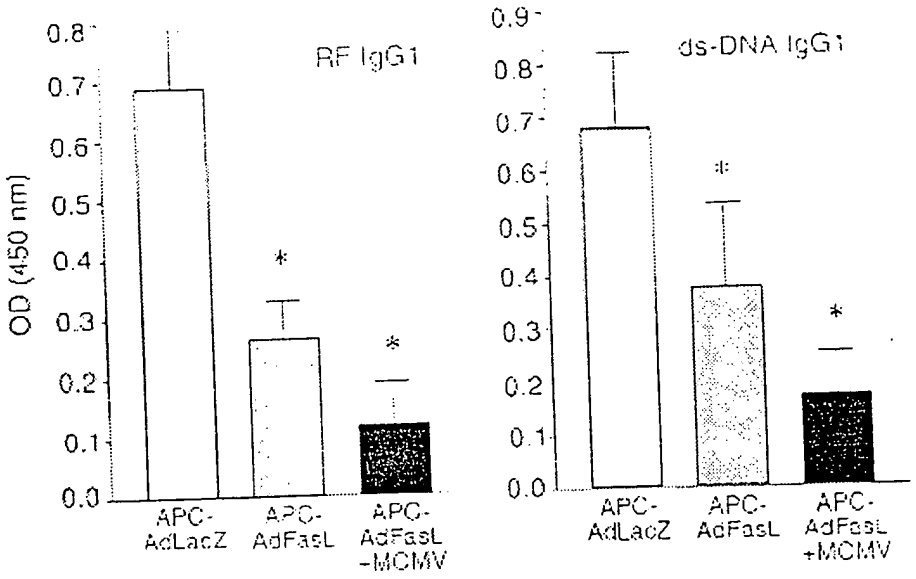

Figure 3

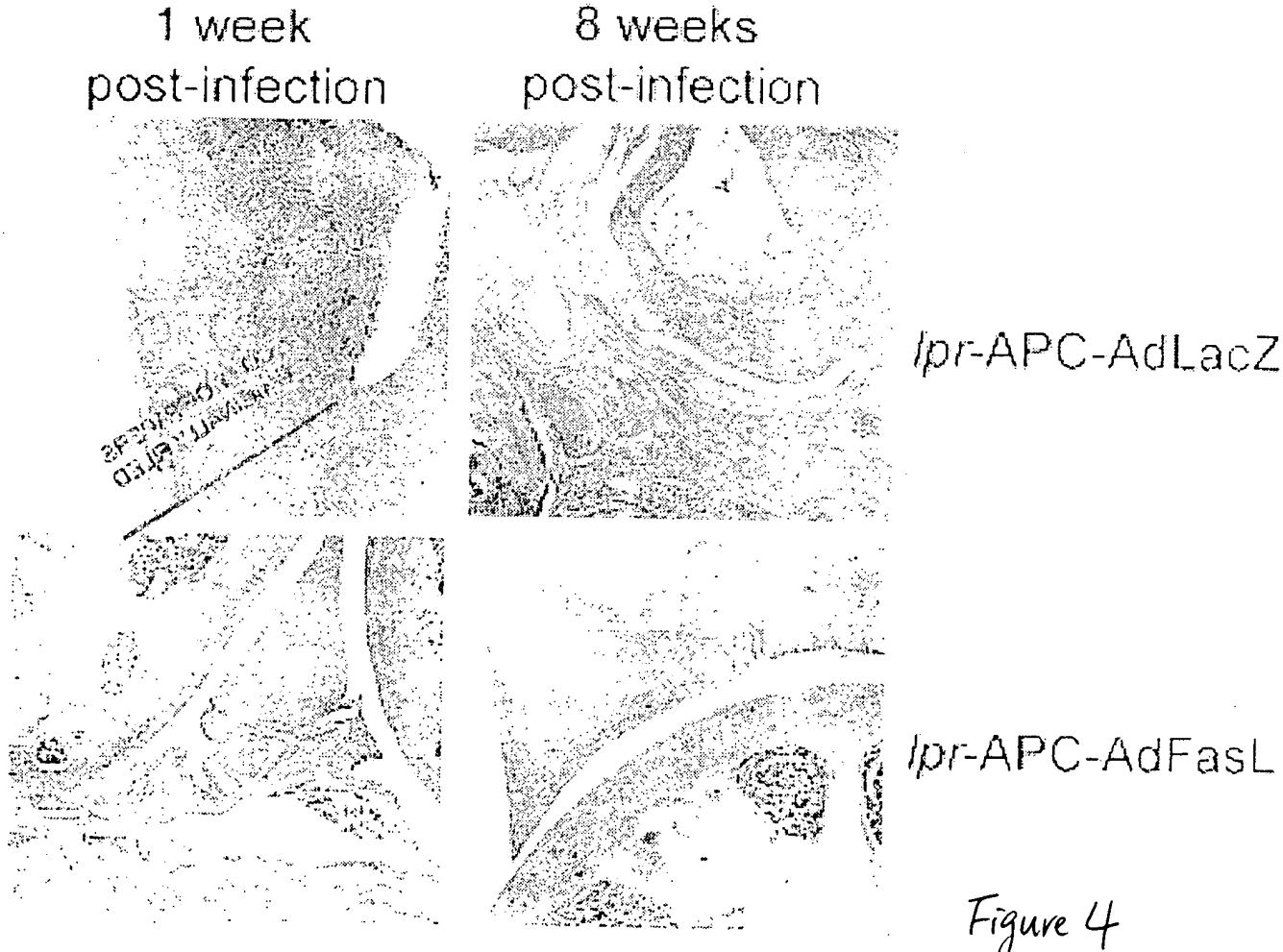

Figure 4